HKUST iGEM 2016

Human practices

Product Design

Background

Aims

Tri-stable switch, a ternary system that has three stable states, in which it has one more equilibrium state than the ordinary bistable switch. Many biological systems are bistable, they make use of the bistability to regulate cell process. Imagine for tristable switch, the output depends on the balance of three signals, such combination could make it a powerful tool that helps in the regulation of biological process like detecting more than two chemicals at the same time and decide which is dominant and make corresponding response and at the same time, it is more sensitive ("Brown: Tri-stable", 2006), when compared to bistable switch.

In terms of biosensor, tristable switch would be an advantage. There were many past iGEM projects in the track of biosensor, for example, the detection of different contaminants like heavy metals, toxic chemicals like mercury and microRNA that are specific to cancer. It would be beneficial to have more states in such signal detection, as different combination of signals in the three-circuit switch could result in more possible output. It is hoped that the tristable switch could benefit the future iGEM teams that it could help as a toolkit for characterization, for example, the promoter strength and the gene-gene interaction. Theoretically the tristable switch could be modified easily by changing the parts, replacing different promoters, in which it could be useful different applications. However, characterization is required for the combination of parts in the switch. Moreover, a kill switch could be included as one of the states in the biosensors for safety purpose.

Next possible usage

Tri-stable switch has several possible usages. Theoretically, it can create 3 separate switches into one stable system. It makes the function to be more efficient since the parts can be changed according to the purpose of tri-stable switch itself. Specific sites need to be added for changing any parts of the construct. The specific sites help to remove the parts that are necessary to be changed and replace it with parts that we want. This way we can obtain the desired switch.

In biocomputing, tri-stable switch can be used in ternary system. One of it is using tri-stable switch as representation of data and instructions. Instead of having only 0 and 1, tri-stable switch can make people have 3 digits which are 0, 1, and -1 that create more complex and balanced code.

However, since biocomputing is not really developed nowadays, it is hard for us to collect the data and find the proof that biocomputing is really one of the possible applications for tri-stable switch. On medical field, nobody have ever done the clinical trial for tri-stable switch as treatments for diseases. In this case, it is not safe to insert tri-stable switch into human body, since mutation might happen. But after tri-stable switch is learned further, people can consider tri-stable switch for these kind of applications. For now, we make tri-stable switch as a biosensor.

In Hong Kong, there were several accidents caused by an infected trees. Even though the government promised to solve this 'urban time bomb', it has not been improved until now. Some accidents caused by falling trees would still happen, especially when the weather is bad. The reasons for not being able to solve it thoroughly is due to the inability of detecting the wounds and infections. Infections would occur when there is injuries, thus, the tree experts have to use quite a number of equipment to detect the wounds. It could be an effective methods if the wounds are large enough to be detected. Unfortunately, infections could also be caused just because of some tiny flaws which are hard to be ferreted out. It would undermine the accuracy of the equipment and prevent the experts from identifying the locations of the wounds.

Aside from the difficulties for detecting fine flaws, fungal infection is also causing problem. Most fungi are associated with plants as saprotrophs and decomposers. These fungi break down organic matter of all kinds, including wood wand other types of plant material. Wood is composed primarily of cellulose, hemicellulose, and lignin. Lignin is a complex polymer that is highly resistant to degradation, and it encrusts the more readily degradable cellulose and hemicellulose. Fungi are among the few organisms that can effectively break down wood, and fall into two main types—brown and white rot fungi.

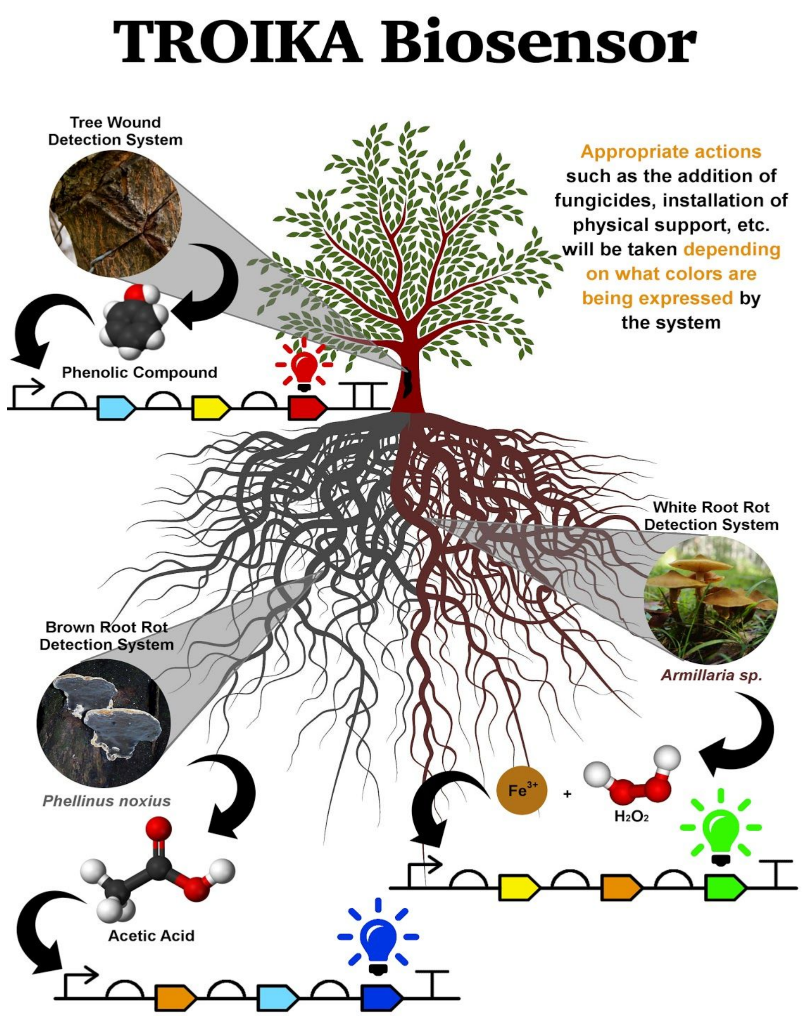
In order to solve this problem to ensure the safety of pedestrians, we would like to develop the tri-stable switch into an useful product which could be able to detect the fine flaws and infections to prevent or at least reduce these accidents.

Key technology

Principle Behind Tristable Switch

Tristable switch, a genetic circuit relies on three chemical inputs and has three outputs. When detected a specific amount of inducer, the corresponding promoter is stimulated, then the protein is synthesised. Until the concentration of another inducer reaches its threshold to alter the state of the switch, protein synthesis remains stable.

Figure 1: Troika Biosensor Mechanism



The three promoters, namely *A, B* and *C*, correspond to three repressors R_A, R_B and R_C, and inducers I_A, I_B and I_C respectively. To spark off the synthesis of Protein A (P_A), I_A combines with repressor R_A, which offsets the transcription of, then followed by the synthesis of Protein P_A, while the other two, B and C are repressed by the corresponding repressors: R_B and R_C. If we want to change the output i.e. from P_A to P_B, a specific amount of I_B is required to alter the state of equilibrium and the state of the circuit.

Assume the strength of promoters *A, B* and *C* are exactly the same. The switch is a potential oscillator, which alters its states sequentially. If there are two inputs, there will be two oscillating outputs, which is the same when there are three inputs.

Advantages of Tristable Switch over Bistable Switch

In terms of information system and biological memory, tristable switch can adopt ternary system. Ternary system, in advance, can have more combination of inputs and outputs than the binary one, which allows the computational system process higher quantity of data and record the corresponding output.

As a biosensor, tristable switch can target more chemicals than bistable switch does. For instance, if you target on one particular fungus, you can detect three detection markers sequentially instead of two. A combination of the promoters and gene-to-be-expressed can be constructed according to the situation. The following application is one of the examples.

Principle Behind Application - Tree diseases detector

Background

In tropical and subtropical area, tree collapse due to fungal infection is commonly seen. Fungi would enter the wounds and root rot, then degrade the internal wood fibres. From the outlook of the tree trunk and branches, it is usually unobservable. One possible way to prevent tree collapse is by diagnosing each of the single tree by a limited number of tree experts. Feasible though, it is by far inefficient and impossible to keep an eye on every tree. Consequences could be severe if those potential danger slip through the net.

How it works

By using the tri-stable switch, we can construct a fungal detector, which senses the wounds and the fungi that destroy the internal structure of the trees.

For the wounds, a wounded tree secrets phenolic compounds i.e. phenols, flavonoids and proanthocyanidins to resist the invasion from pest and the attacking agents (Vek et al., 2012). When phenolic compounds are produced, the switch is induced to produce chromoprotein in the sap, which flow through the surface of the bark as a symptom of fungus infection.

For the fungi, brown root rot disease and white rot are our targets. Brown-rot fungi break down cellulose and hemicellulose, which are the component of trunks. Making use of hydrogen peroxide (H_2O_2) produced after decaying hemicellulose, fungi undergo oxidative process to convert cellulose to acetic acid (Deacon, 2006). The infection usually starts from the root to the lower trunk, which blocks the translocation and transpiration of nutrients and water, subsequently the tree dies due to dehydration and nutrient loss. For white rot fungi, not only do they decay cellulose and hemicellulose, but also lignin. It turns wood from brown to pale, light-weighed, and make it to stringy texture. By detecting compound like Fe^{3+} and H_2O_2, which are produced during the degradation of lignin, certain chemical can be produced to kill the fungi (Have&Teunissen, 2001).

Infected by Two Diseases

Unfortunately, if the tree is infected by two diseases, the sensor would first detect the dominated flaws, then treat it. After that, it moves on detecting another one to ensure the problems are tackled one followed by another. This is more convenient to keep track on the situation of the trees.

Accuracy of the sensor with tristable switch

The switch will only respond to specific external inputs (in this case maybe Urushiol as the phenolic compound, benzaldehydes as the chemical released when white rot fungi act on lignin), therefore the diseases and problems can be spotted accurately.

Making tristable switch be a non-intrusive sensor

As mentioned before, the sensor is engineered into a liana plant, but not the tree itself, which makes it a non-intrusive one.

Liana, a climbing plants, shares the same habitat as the fungus in temperate forest (Schnitzer et al, 2014). It goes up along the tree trunks and branches as it grows in order to obtain sunlight. Reaching every single corner of the tree, it can carry the sensor after we implant it into the leaves of Liana and check the tree effectively.

Product line

Our product, Tristable Switch, is more advantageous when it comes to biosensors and also the stability of the switch. In terms of the design, Tristable Switch has one more stable point than bistable switch. It has three stable/equilibrium points instead of two, which is more informative. In terms of biosensors, Tristable Switch gives more possibilities and are easier to be manufactured. Only by changing the promoters of the switch, a huge range of biomolecules could be detected, and more possible biosensors could be made. Due to the tri-stability of the switch, most abundant biomolecules could be selected out of a group of three different biomolecules.

An easier way to discover problems in trees:

By using tree biosensor, an easier way is given to investigate some of the problems that may occur in trees. White/brown rot fungal infections can be detected by looking out for definite signals and chemicals. Not only professionals can discover the problems , but also public with training.

A more detailed checking than by man:

The tree biosensor is cloned in liana and is grown surrounding the tree (Rabenantoandro et al.,2008) . It can detect small flaws which is undetectable by or easily missed by human eye.

Can be manufactured easily:

Manufacturing a tree biosensor requires only cloning the tristable switch in to liana. This requires much less time and expenses than training an experienced professional in tree. In addition, it is also more environmentally friendly than using pesticide.

Product Description

This biosensor is able to detect three different tree flaws and diseases, including wounds, brown root disease and white root disease. For each problems, the sensor will show different signals. the sensor will only show one signal at a time, which reveals the most threatening problem among the three. the product is a form of engineered liana plant. Plant the plant near the target and it will grow and climb the tree, no extra care for the plant is needed. The plant will surround the tree normal liana leaves appear green. when the tree shows flaws, the liana sap near the wound will show orange. when the tree acquires brown root disease, the sap shows red. when the tree got white root disease, the sap shows blue. remove the plant immediately if the the liana leaves has mostly covered the tree's leaves.

Windows of opportunities

As the gene circuits of the fungal detector is made of Biobricks, the fungal detector can perform different functions by changing the parts. We can also tailor-made a fungal detector to detect other types of fungi and wounds for consumers. Also, a tool-kit for promoters that sense for different fungi and specific restriction sites for swapping the parts in and out will be provided to consumers in accord with their needs. As the parts are interchangeable, we can set up a library for consumers, inventors and scientists, who can get access and exchange their parts to improve sensor performance. The reusable parts can reduce the average cost of the switch, and thus reduces the invention cost. We could also rely on a machines and programmes such as DNA amplification by PCR machine to produce desired parts in large quantities automatically.

Economically, the fungal detector is beneficial to both human and ecosystem. As the fungal detector helps us monitor the trees, this can prevent accidents happened so as to protect public property and human life. Furthermore, the detector can reduce human

resources and money required for care-taking. The cost of maintenance can be decreased as well.

Besides, the fungal detector is propitious for environment as it can reduce herbicides, and fungicides usage. Fungicides can diminish biodiversity, disrupt natural biological equilibrium and contaminate other non-targeted areas. The fungal detector can detect fungi and wounds, then secrete chemicals to heal wounds and kill fungi. Therefore, less or ever no fungicides are needed for tree protection and maintenance. The fungal detector can help us protect heritage trees. Old and valuable trees can be monitor by the sensor. Once the product is applied, no refill is needed. Therefore, natural resources can be saved.

The fungal detector could be effective in monitoring the health condition of trees, but it may lead to safety concerns. Firstly, the behavior of synthetic biological systems is uncertain and unpredictable. In legal aspect, it is important for us to ensure the benefits from this developing field of science, identify appropriate ethical boundaries and minimize the risks. However, risk assessment protocols of the fungal detector have not yet been developed to assess the potential ecological risks associated with synthetic biology and the product. Also, There could be a problem of dual use as some bioterrorists may use the sensor as a bioweapons. The fungal detector originally produced for monitoring trees, but it has the potential to be a bioweapon as bio- terrorists can use it for toxic production or infectious fungi production.

Challenges

Social

Applications of biotechnology are diverse and far-reaching, with synbio being a new paradigm in the biotechnology revolution. However, its popularity has not reached a level whereby the general public are willing to expose themselves to synthetic biological products with open arms - most of which are due to public concerns regarding the their safety when being applied for day-to-day use. Following the guidelines and safety laws that are adopted by the European countries or the United States is suggested, since those guidelines have

been revised regularly and are more suitable for a research environment in which researchers can enjoy greater flexibility in their research whilst ensuring the citizen's safety (Carter et al., 2014).

Meanwhile, the government is investing tremendous efforts and funds (HK$500 billion annually) to promote STEM (Science, Technology, Engineering and Mathematics) education and encourage young generations to pursue the study of these subjects. Hence, public knowledge regarding areas such as synthetic biology is predicted to grow on further in the long term ("Innovate for the", 2016).

Technological

Few technological challenges have been identified from the tri-stable biosensor, the first being its sensitivity. The system only detects a specific range of phenolic compound concentration; and hence is incapable of detecting concentrations specifically and accurately that are out of range. Some phenolic compounds can remain undetectable, especially ones with structural complexities whereby the phenolic group is restricted within an enclosed region in the overall structure. In addition, the amount of phenolic compounds produced by tree wounds too varies according to the plant species. Similarly, there is a need for clarifying the system specificity towards different nutrients in the tree sap overflow that is caused by fungal infection. Breaks or imperfections in the tree's microflora can be caused by other non-fungal factors such as insects, birds or even humans. These are normal occurrences that cause the overflow of tree sap, despite not posing any harm to rigidity. Further research is essential to find ways in making the system capable of differentiating these situations.

Currently, our product only constitutes the tri-stable switch as a sensor. However, it may be developed to a device that can also kill the fungi or heal the wounds of the tree stem and roots. If the product is to be developed for real-time use, the ability for it to distinguish between different fungi is essential, as each fungal species has different resistances towards the kill-switch chemical.

Another challenge is the delivery of the tri-stable sensor. While using the climbing Lianas for host of the device has been considered, they also need time to grow and climb

up trees that are multiple meters high. Given that Lianas reproduce quite easily, releasing them as a host for the tri-stable sensor can lead to environmental concerns that will be discussed later on in the report.

Legal – Bureaucracies and Safety

Currently, Hong Kong Government establishes its first Biodiversity Strategy and Action Plan, with enhanced measures on protecting ecological conservation zone and species. Though this might mean more recognition for our project, it could also mean that the regulations regarding environmental control will be far stricter than it is now. Innovation and Technology Bureau (ITB) will make strenuous efforts to coordinate the work of universities (“Innovate for the”, 2016).

The tri-stable switch is an innocuous circuit, containing genes that can be found in many other organisms and is therefore harmless on its own. In terms of safety regulations, barely is there any law regarding synbio products, as most of them marketed today are in the form of mainstream chemical products rather than the genetic device itself – only the production method is different. What the government concerned about, however, is the environmental safety protocols that regulate the release of these devices into nature. One of these is the Cartagena protocol on biosafety – an international treaty governing the movements of living modified organisms (LMOs) resulting from modern biotechnology from one area to another (“About the Protocol”, 2012). Most products of synthetic biology lie outside this protocol and addressing them requires updates to risk assessment and management approaches that are handled by the Convention of Biological Diversity. This will slow down the product development especially if the evaluation on our biosensor needs to be done in multiple places.

Environmental and Ethical Issues

Related to biosafety measures, the tri-stable biosensor can also raise some environmental and ethical issues. When the device is released into public soil or trees in the wild, the trait can be distributed rapidly across the host population, or even across an

entire species, which can be achieved rather quickly in species with a short reproductive cycle. If especially some fungicidal behaviors were to be introduced into the device, the population of particular fungi species would fall rapidly. The host organism might also have some interaction with the rhizosphere of the tree and disrupt it. In short, the effects towards the ecological balance of the environment are still unknown.

Another ethical concern is that tri-stable biosensor could potentially replace other existing similar products and hence threatens the livelihood of local biosensor or fungicide producers ("NGOs highlight synthetic", 2016). There is a chance that when further development is conducted, our device might be applicable for other situations relating to fungal infection as well, especially those in the scope of agriculture. This would then break through a much larger market, and such protests may arise.

These challenges are inevitable given that the project is still at its very early stage of development. However, further fine-tuning of the device are under continuous revision.

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